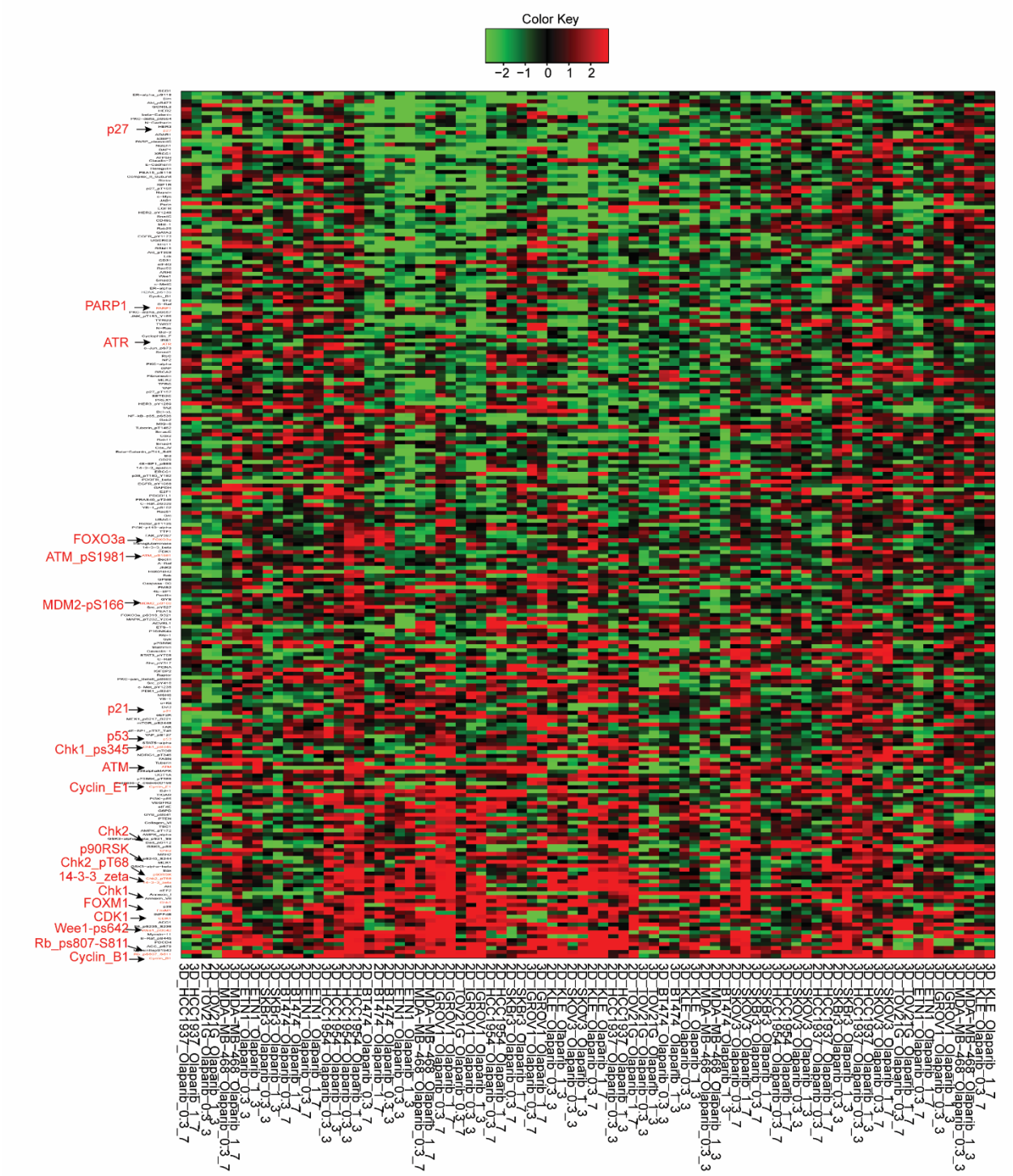
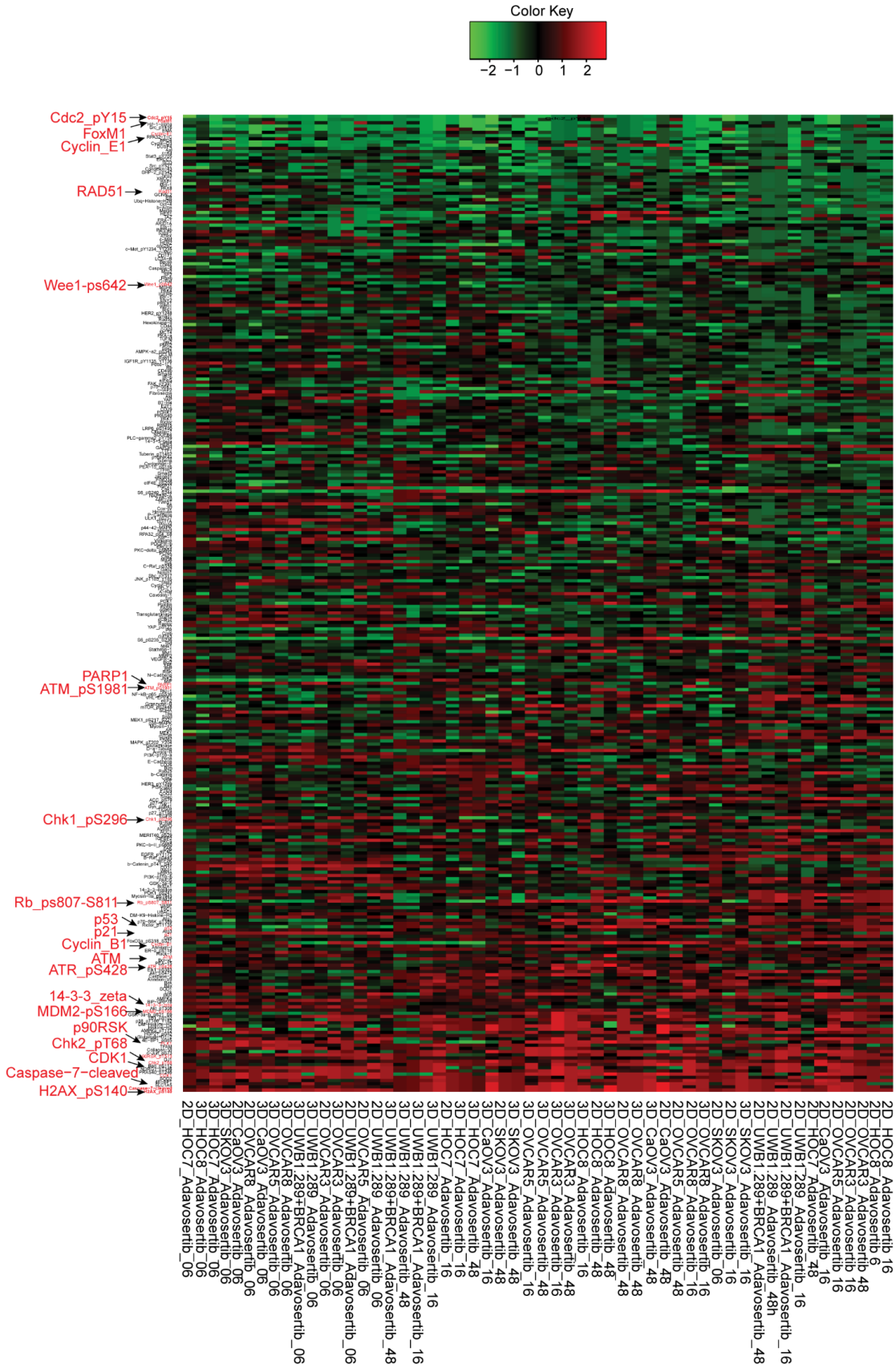


Figure S1, related with Figure 1. Effect of olaparib on protein expressions across cell lines. Five breast cancer cell lines (BT474, HCC1954, HCC1937, MDA-MB-468 and SKBr3), three ovarian cancer cell lines (IGROV-1, TOV21G and SKOV3) and two endometrial cancer cell lines (KLE and ETN-1) were cultured in Matrigel (3D) or monolayer (2D), treated with olaparib at indicated concentrations for each line for 2D and 3D conditions or DMSO for 3 or 7 days, and

protein lysates analyzed for 218 total and phosphoproteins by RPPA. The sample IDs in the heatmap are presented as: dimension (2 or 3D) _cell line name_ concentration (μM) _days.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3D_OVCAR5_Adavosertib_06
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2D_OVCAR3_Adavosertib_48
2D_OVCAR3_Adavosertib_16
2D_HOC8_Adavosertib_16

Figure S2, related with Figure 1. Effect of WEE1 inhibitor on protein expression.
Nine ovarian cancer cell lines (HOC8, OVCAR8, CAOV3, UWB1.289+*BRCA1*, UWB1.289, OVCAR5, HOC7 and SKOV3) were cultured in 3D or monolayer 2D, treated with adavosertib at an IC_{50} concentration determined experimentally for each line for 2D and 3D conditions or DMSO for 6 hr, 16 hr, or 48 hr, and protein lysates analyzed for 305 total and phosphoproteins by RPPA. The sample IDs in the heatmap are presented as: dimension (2 and 3D) _cell line name_drug_hr.

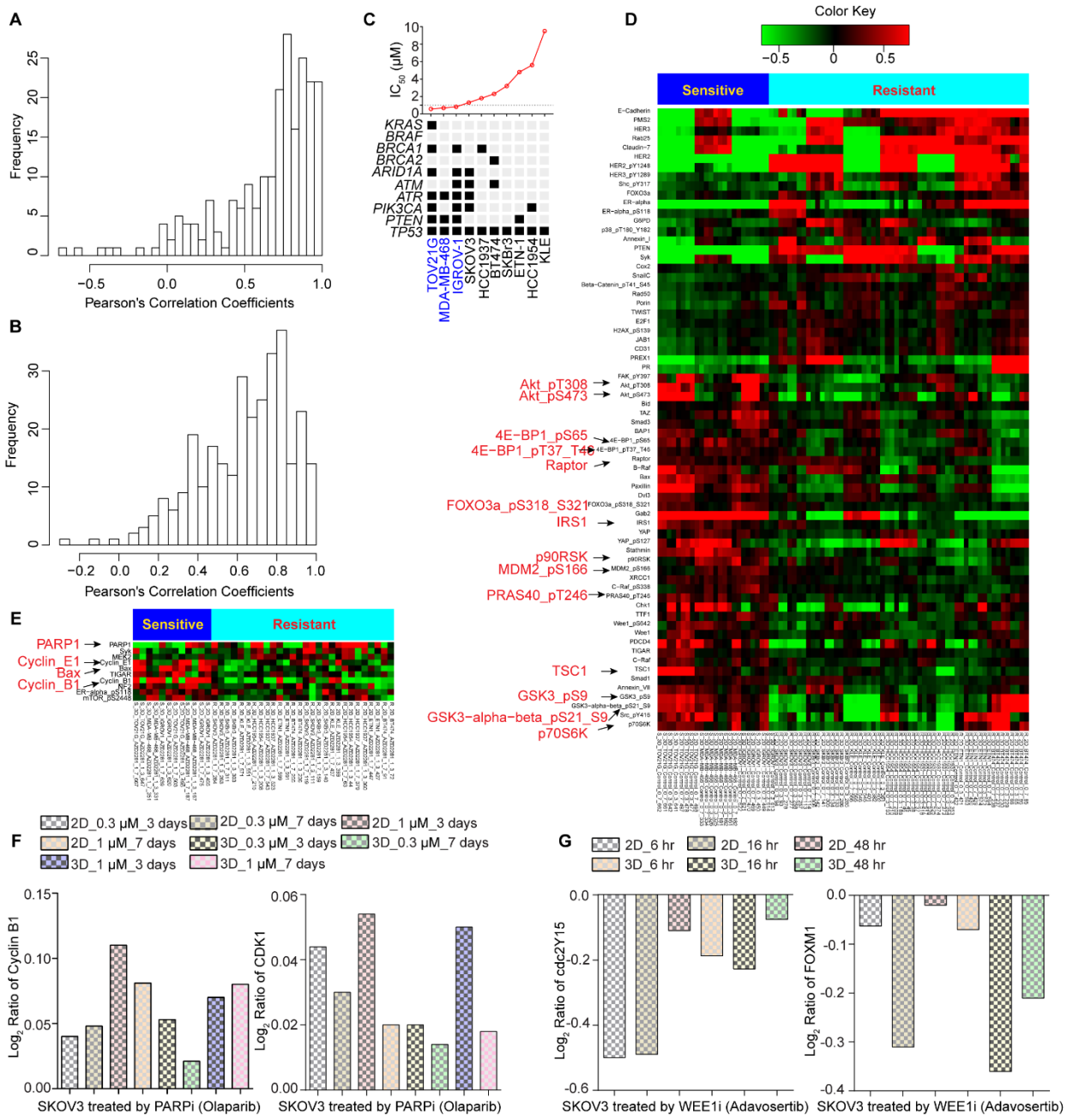

Figure S3, related to Figure 1. Effects of PARPi and WEE1i on protein expression.

(A) Pearson's correlation coefficient analysis of RPPA analysis of protein expression between olaparib treated 2D and 3D cultures.

(B) Pearson's correlation coefficient analysis of RPPA analysis of protein expression between adavosertib treated 2D and 3D cultures.

(C) IC_{50} values of olaparib (top) and selected mutations (middle) in 10 cancer cell lines treated with olaparib. Black indicates a mutation within the respective gene, gray indicates no mutation.

(D) Significant differences in protein levels between sensitive and resistant cell lines based on IC_{50} in (C) were used to select proteins for evaluation by heat maps (Student's *t* test with

Bonferroni adjusted p value of < 0.05). The sample IDs in the heatmap are presented as: dimension (2 and 3D) _cell line name_drug_days_sample ID.
(E) Significant differences between sensitive and resistant cell lines after olaparib treatment were used to select proteins for evaluation by heat maps (Student's *t* test p<0.05). The sample IDs in the heatmap are presented as: dimension (2 and 3D) _cell line name_drug_days_sample ID.
(F, G) Protein expression changes in SKOV3 based on RPPA data from PARPi (F) or WEE1i (G) monotherapy.

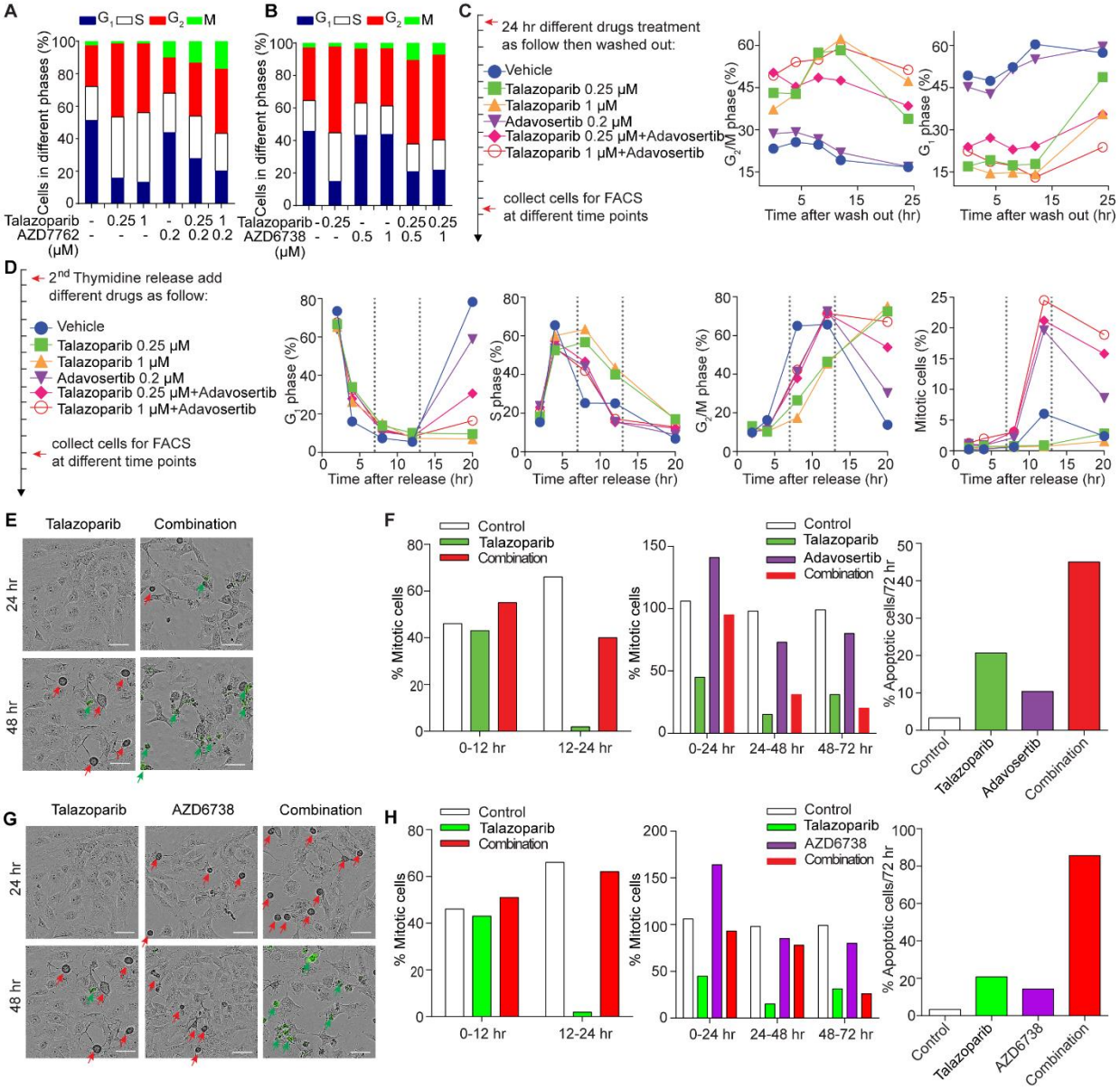

Figure S4, related with Figure 2. Effect of PARPi and WEE1i, ATRi or CHK1/2i on cell cycle progression and cell death.
(A) OVCAR8 were treated with talazoparib, AZD7762 (CHK1/2 inhibitor), or the combination for 24 hr and subjected to dual pH3 (mitotic cells) and PI (DNA content) flow cytometric analysis.

(B) OVCAR8 were treated with talazoparib, AZD6738 (ATR inhibitor), or the combination for 24 hr and subjected to dual pH3 (mitotic cells) and PI (DNA content) flow cytometric analysis.
(C) OVCAR8 were treated by mono or combination treatment of talazoparib and adavosertib for 24 hr at the indicated concentrations, drugs washed out, cells re-cultured and cell cycle progression monitored by flow cytometry. Proportion of G_2-M phase, G_1 phase were calculated.
(D) OVCAR8 were synchronized using double thymidine block. After thymidine removal, DMSO, talazoparib, adavosertib, or the combination was added at concentrations as indicated. Cell-cycle (PI) and mitotic (pH3) profile were measured at indicated time points by FACS. Proportion of G_1 phase, S phase, G_2-M phase, and M phase cells were calculated. The left dashed line indicates the separation time of G_0/G_1 and S phase, and right dashed line indicates the separation time of S and G_2-M phase.
(E-F) OVCAR8 were either exposed to DMSO (control) or talazoparib (0.5 μM), adavosertib (0.5 μM), or combination with medium containing IncuCyte cleaved caspase-3/7 reagent (green). Scale bar, 50 μm. Representative images at different time points treated with Talazoparib or combination (E). Red arrowheads indicate mitotic cells, green arrowheads indicated apoptotic cells. Percent of cells entering mitosis or apoptosis in different periods after indicated treatment (F). At least 300 cells were scored per data point.
(G-H) OVCAR8 were either exposed to DMSO (control) or talazoparib (0.5 μM), AZD6738 (2.5 μM), or combination with medium containing IncuCyte Cleaved Caspase-3/7 reagent (green). Scale bar, 50 μm. Images represent different time points treated with talazoparib or combination (G). Red arrowheads indicate mitotic cells, green arrowheads indicate apoptotic cells. Percent of cells entering mitosis or apoptosis in different periods after indicated treatment (H). At least 300 cells were scored per data point.

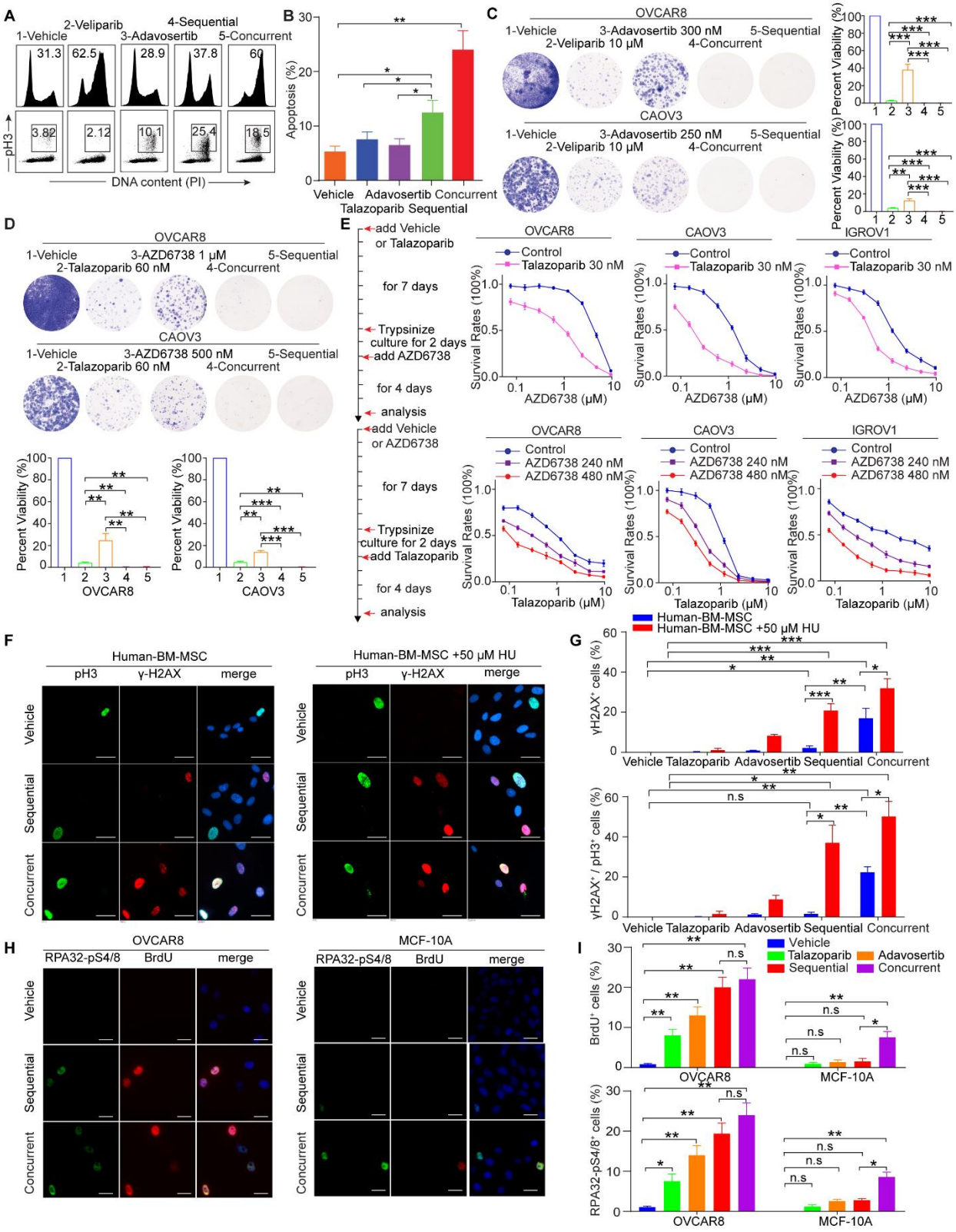

Figure S5, related with Figure 5 and Figure 6. Sequential treatment is effective in tumors with less toxicity in normal cells in vitro.

(A) OVCAR8 were treated with DMSO, veliparib (30 μM), adavosertib (200 nM), sequentially or concurrently as in Figure 5A and subjected to dual pH3 (mitotic cells) and PI (DNA content) flow cytometric analysis.
(B) OVCAR8 were treated with DMSO, talazoparib (500 nM), adavosertib (200 nM), sequentially or concurrently for 24 hr and subjected to Annexin V + PI flow cytometric analysis.
(C) Cells were treated with different drugs for 14 days, except for sequential therapy that consisted of PARPi for 7 days followed by the other drug for 7 days. Representative photos of colony formation stained with crystal violet for each treatment.
(D) Cells were treated with different drugs for 14 days, except for sequential therapy that consisted of PARPi for 7 days followed by the other drug for 7 days. Representative photos of colony formation stained with crystal violet for each treatment. Note: sequential treatment of PARPi and adavosertib or AZD6738 was in the same experiment as Figure 5G, so the vehicle and talazoparib data in Figure 5G is repeated here.
(E) Cells were treated with DMSO or 30 nM talazoparib for 7 days followed by AZD6738 (upper), or treated with vehicle, 240 nM AZD6738 or 480 nM AZD6738 for 7 days followed by talazoparib (lower).
(F) Cells were treated with DMSO, talazoparib, adavosertib, sequentially or concurrently as in Figure 5A in the absence or presence of 50 μM HU for 24 hr and analyzed for γH2AX, pH3 by immunostaining. Scale bar, 20 μm.
(G) Quantification of pH3 and γH2AX positive cells treatments as described in (F). For each condition, at least 300 cells were counted.
(H) Cells were cultured in 10 μM BrdU for 36 hr, treated with DMSO, talazoparib, adavosertib, sequentially or concurrent treatment as in Figure 5A for 24 hr, and analyzed for native BrdU (ssDNA) and RPA32-pS4/8 by immunostaining. Scale bar, 20 μm.
(I) Quantification of native BrdU (ssDNA) and RPA32 pS4/8 positive cells with treatments as in (H). For each condition, at least 300 cells were counted.
Data across panels are mean±SEM and Student's t test: *$p<0.05$, **$p<0.01$, ***$p<0.001$, n.s.: not significant.

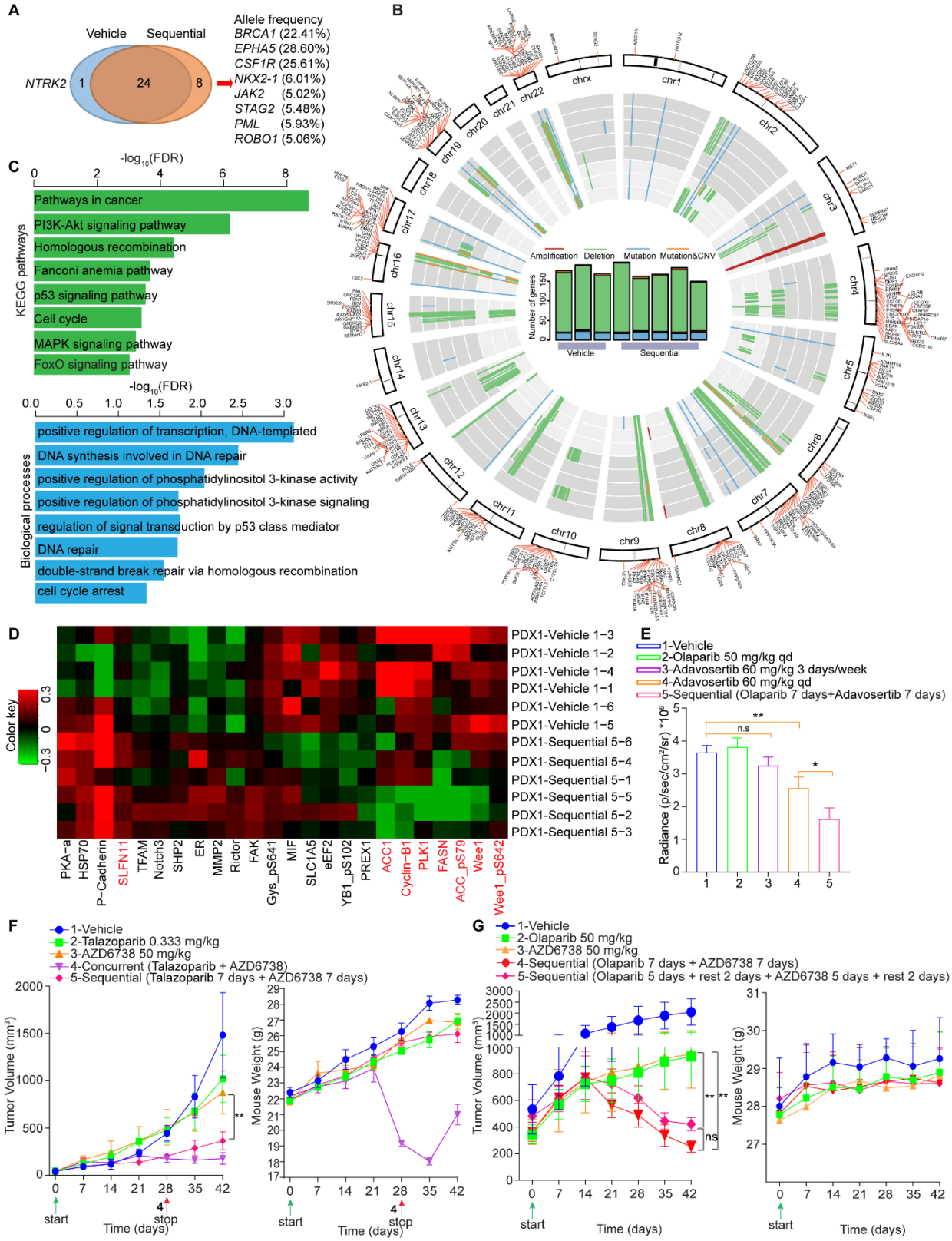

Figure S6, related with Figure 8. Sequential administration of PARPi and WEE1i is effective and tolerable in ovarian cancer models.

(A) Mutation analysis between vehicle and sequential treated tumor samples at 105 days based on T200.2 exome sequencing.
(B) Mutations and copy number variation in different chromosomes between vehicle and sequential treated tumor samples at 105 days based on T200.2 exome sequencing.
(C) Function enrichment analysis based on CNV changes in sequential treatment compared to vehicle. upper: Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis, lower: Biological processes in Gene Ontology (GO) analysis.
(D) RPPA analysis in both vehicle and sequential PARPi and WEE1i treated tumor samples. Student's *t* test was used to compare protein expression in treated samples with those in controls. p of < 0.05 was used as cutoff to select significantly deregulated proteins for the heatmaps.
(E) ID8 i.p injection model was treated by indicated therapies. Bioluminescence was determined on day 28. Data are mean±SEM. p values were determined by one-way ANOVA. *p<0.05, **p<0.01, n.s: not significant.
(F) PDX2 was treated as indicated. The 4^{th} group was stopped on day 21 due to weight loss. Average tumor volume±SEM for each cohort is displayed. p values (sequential compared with AZD6738 monotherapy) were determined by one-way ANOVA (left). **p<0.01. Average mice weight±SEM for each cohort is displayed (right). Note: This model was done together with adavosertib, so it shares the vehicle and talazoparib treatment and mice weight curves with Figure 8B.
(G) PDX1 was treated as indicated. Average tumor volume±SEM for each cohort is displayed. p values (compared with talazoparib monotherapy) were determined by one-way ANOVA (left). n.s: not significant, **p<0.01. Average mice weight±SEM for each cohort is displayed (right).